available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/peptides

A peptide inhibitor of MurA UDP-N-acetylglucosamine enolpyruvyl transferase: The first committed step in peptidoglycan biosynthesis

José Molina-López [a,1], *François Sanschagrin* [b], *Roger C. Levesque* [b,*]

[a] *Departamento de Salud Pública, Facultad de Medicina, Universidad Nacional Autónoma de México, Mexico*
[b] *Centre de Recherche sur la Fonction, Structure et Ingénierie des Protéines, Pav. C.-E. Marchand, Faculté de Médicine, Université Laval, Sainte-Foy, Québec, Canada G1K 7P4*

ARTICLE INFO

Article history:
Received 22 June 2006
Received in revised form
22 August 2006
Accepted 23 August 2006
Published on line 9 October 2006

Keywords:
MurA
Phage display
UDP-N-acetylglucosamine
Peptide inhibitors

ABSTRACT

The MurA enzyme from *Pseudomonas aeruginosa* was purified to homogeneity and found to be biologically active as a UDP-N-acetylglucosamine (UNAG) enolpyruvyl transferase in a coupled enzyme assay where ATPase activity was measured by the release of inorganic phosphate. A microtiter plate assay coupled to competitive biopanning using the UDP-N-acetylglucosamine was used to screen 10^9 C-7-C and 12-mers peptides from phage display libraries. From 60 phage-encoded peptides identified after the fourth round of biopanning, deduced amino acid sequences were aligned and two peptides were synthesized and tested for inhibition of the MurA-catalyzed reaction. The PEP 1354 peptide inhibited the ATPase activity of MurA with an IC_{50} value of 200 μM and was found to be a competitive inhibitor of UNAG. The pre-incubation of MurA with inhibitor indicated a time-independent inhibition. This time-dependent inhibition is the first report of peptide inhibitors of MurA, which represent the scaffold for the synthesis of inhibitory peptidomimetic molecules.

1. Introduction

Peptidoglycan is an essential cell wall component of most bacteria and their biosynthetic pathway is a complex of two-stage process [18]. The first stage occurs in the cytoplasm, and consists in the formation of the monomeric building block N-acetylglucosamine-N-acetylmuramyl pentapeptide. This first committed step in this pathway is the transfer of an enolpyruvate residue from phosphoenolpyruvate to position 3 of UDP-N-acetylglucosamine [5]. This reaction is catalyzed by MurA enzyme (Fig. 1). This is followed by a MurB-catalyzed reduction of the enolpyruvate moiety to D-lactate, yielding UDP-N-acetylmuramate. A series of ATP-dependent amino acid ligases, MurC to MurF catalyze the stepwise addition of amino acids to the pentapeptide side-chain to form UDP-N-acetylmuramyl pentapeptide. MurA to MurF cascade is ubiquitous to both Gram-positive and Gram-negative bacteria, and has no homologue in mammalian cells [19].

The inhibition of one of MurA through MurF enzymes leads to loss of cell shape and integrity followed by bacterial cell lysis and death [8,14]. However, enzymes implicated in this pathway are not inhibited by known antibacterial agents, except for MurA, inhibited by fosfomycin [15,25] in which the active ingredient of Monurol forms a covalent adduct with a cysteine residue, Cys^{115} (numbering according to MurA from *Escherichia coli*). Cys^{115} is located in a solvent exposed loop that undergoes

* *Corresponding author*. Tel.: +1 418 656 3070; fax: +1 418 656 7176.
E-mail addresses: joseml@servidor.unam.mx (J. Molina-López), fsanscha@rsvs.ulaval.ca (F. Sanschagrin), rclevesq@rsvs.ulaval.ca (R.C. Levesque).
[1] Permanent address: Av. Universidad No. 3000, Ciudad Universitaria, Colonia Copilco Universidad, Mexico D.F., C.P. 04510, Mexico.
0196-9781/$ – see front matter
doi:10.1016/j.peptides.2006.08.023

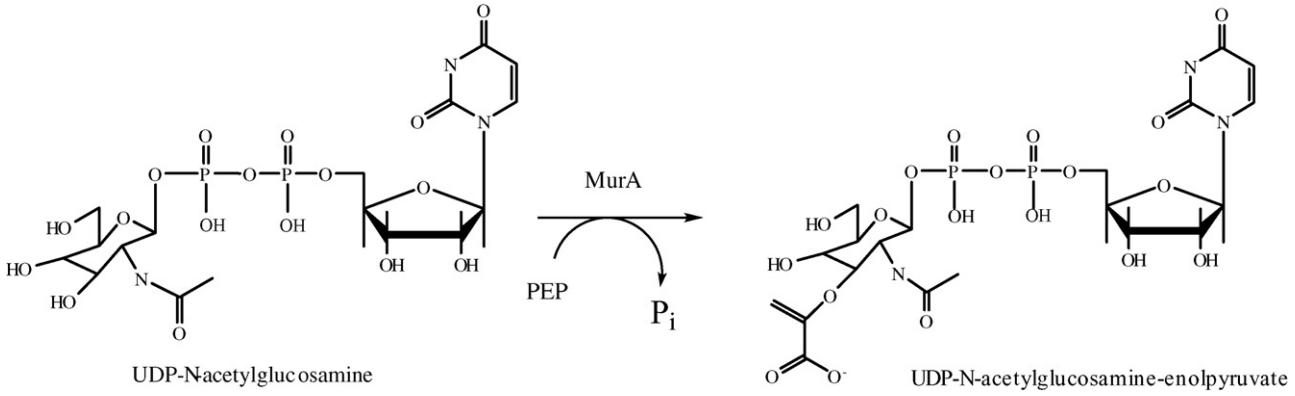

Fig. 1 – Schematic of the first step of peptidoglycan biosynthesis where MurA, a UDP-N-acetylglucosamine enolpyruvyl transferase, catalyzes the enolpyruvyl transfer reaction from enolpyruvate to uridine 5′-diphospho-N-acetylglucosamine.

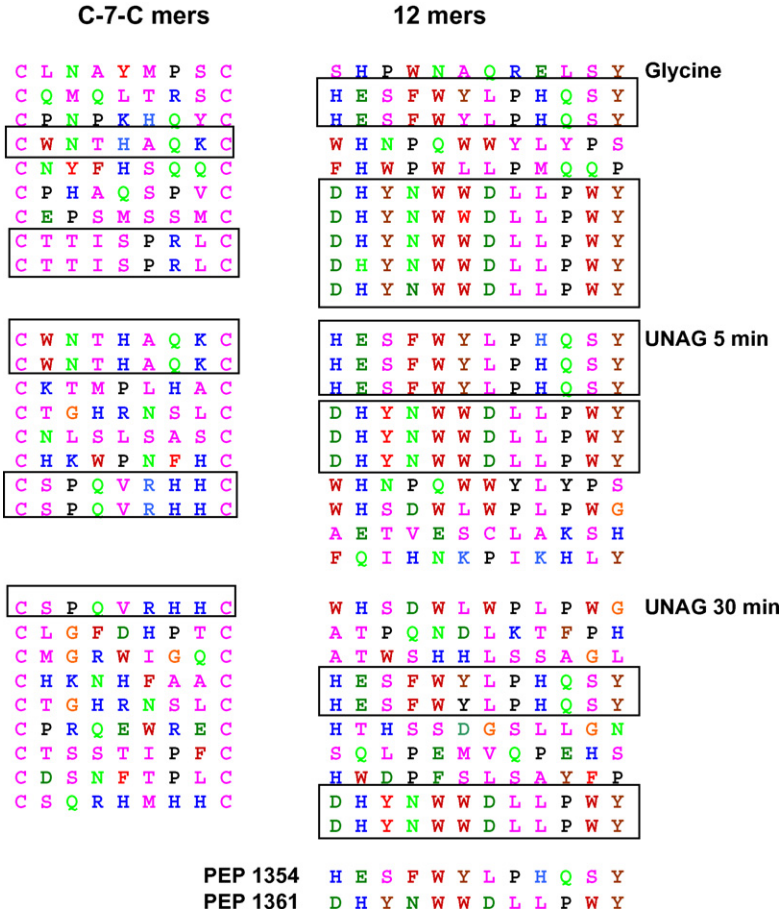

Fig. 2 – Consensus peptide sequences obtained from screening the PH.D.-C-7-C and 12-mers phage-encoded peptide libraries against MurA. The data were compiled from the sequencing of 60 eluted phages after the fourth round of competitive biopanning. The two peptides synthesized PEP 1354 and PEP 1361 and containing consensus amino acid sequences are shown below the alignment. Acidic amino acids (D, E) are in green, polar amino acids (Q, N) are in light green, basic amino acids (K, R, H) are in blue, hydrophobic amino acids(I, L, M, V) are in pink, hydrophobic aromatic amino acids (F, Y, W) are in red, small amino acids (A, S, C, T) are in pink, G (tiny amino acid) is in orange and P (leading to turn formation is in black (classified according the Venn diagram for defining the relationships between amino acids). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)